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THE ORIENTATION OF CELLULOSE IN THE SECONDARY WALL OF TRACHEARY CELLS

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With plates 206–208 and three text figures

INTRODUCTION

Much attention has been focused, in recent years, upon the study of the arrangement of chain molecules, micelles, and fibrils in the cell walls of the cotton hair, bast fibers, tracheids, and other types of commercially important fibers. Various methods of studying the arrangements of the structural units are employed by different investigators:

1. The study of visible fibrils and striations, based upon the assumption that the long axis of the micelles is oriented parallel to these structures.

2. The study of pit-orifices and of mechanically induced cracks, based upon the assumption that these structures are oriented parallel to the fibrillar axis.

3. The study of extinction angles, of dichroism, and of other phenomena in polarized light.

4. The study of X-ray diagrams.

Each of these methods yields significant data under favorable circumstances, but each is subject to serious limitations when applied to miscellaneous types of cell walls.

Even when coarse aggregations of fibrils and striations are clearly visible in surface views of unswollen secondary walls, it frequently is difficult to determine with certainty, whether a specific orientation occurs throughout the wall or in one of its layers only. Swelling the wall to reveal its finer structure is effective in dealing with the broad central layers — provided allowances be made for distortions due to longitudinal contraction and lateral expansion — but such treatments commonly disrupt and conceal the structure of the tenuous inner and outer layers.

In the case of thick secondary walls of the 3-layered type, the long axis of the slitlike pit-orifices is oriented parallel to the fibrillar axis of the central layer, but affords no evidence regarding the fibrillar arrangements of the inner and outer layers. In thin-walled cells, the orifices of the pits commonly afford no clue regarding the fibrillar orientations of any of the layers. Similarly, mechanical cleavages or seasoning cracks may afford valuable evidence in the case of layers which have pronounced radio-longitudinal or radio-helical porosities, but are difficult to interpret accurately in the case of other layers.

Phenomena visible under the polarization microscope are significant where material can be oriented so that the polarized light passes through single layers; but accurate interpretations are difficult where the light passes through several superimposed layers of varying thickness and of different fibrillar orientations. Thus, in the case of transverse sections of 3-layered secondary walls, it is possible to demonstrate that the fibrils of the central layer are, on an average, oriented more nearly parallel to the long axis of the cell than in the case of the inner and the outer layers; but it is difficult to determine in longitudinal sections whether the orientation of the latter layers is actually at right angles to the long axis of the cell or at some intervening angle.

Similar obstacles must be overcome in the interpretation of X-ray diagrams where the rays pass through a complex of layers of markedly varying thickness and of very different structural orientations.

In view of such technical difficulties as these, it is not surprising to find many contradictory statements in the literature concerning the structural arrangement of cellulose in the walls of specific cells. It obviously is essential to develop a technique which will enable the investigator to trace the details of fibrillar orientation throughout each of the successively formed parts of the secondary wall. The method recorded in the following pages appears to be of considerable value in the study of lignified tissues.

METHOD OF DEMONSTRATING THE FIBRILLAR ORIENTATION IN LIGNIFIED WALLS

When sections of lignified tissues are chlorinated, rinsed in 95% ethyl alcohol, treated with dilute ammonia in strong ethyl alcohol, rinsed in alcohol, chlorinated, rinsed in alcohol, stained in a 2–4% aqueous solution of iodine-potassium iodide, and finally mounted under a cover glass in a drop of 60% sulphuric acid, dark brown crystals of iodine form within the layers of the secondary wall. These crystals aggregate in slender, elongated, crystalline complexes which vary in size and number, depending upon the duration and the intensity of the successive treatments and upon other factors. The crystals evidently originate within the elongated interstices of the cellulosic matrix and are oriented parallel to the long axis of the fibrils of cellulose. The crystals, or crystalline aggregates, are so conspicuous and so clearly visible, microscopically, that it is possible not only to detect such major variations in orientation of the cellulose as occur in passing from layer to layer of the secondary wall, but also to observe such fluctuations in orientation as occur within the limits of a single layer.

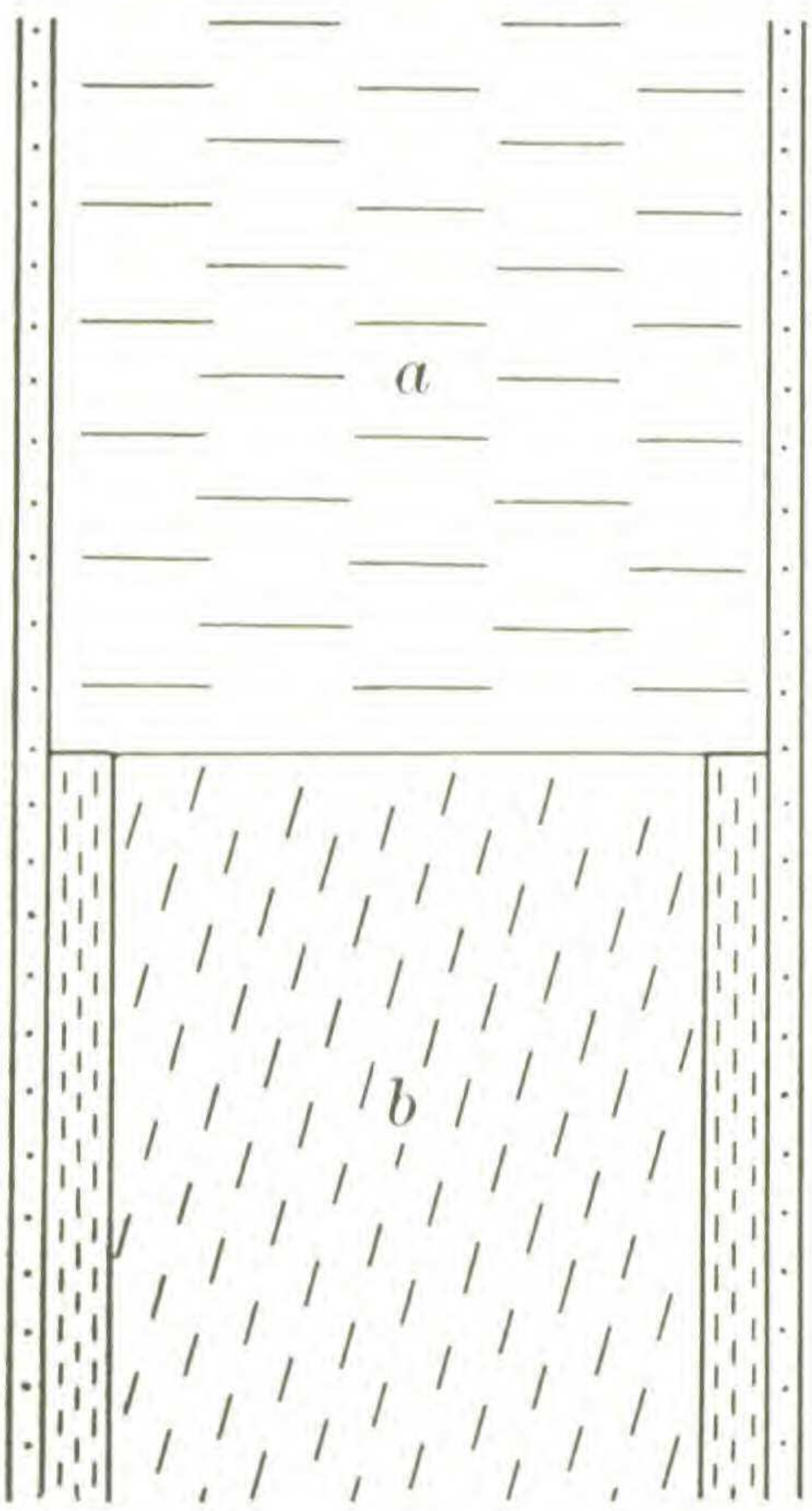
ORIENTATION OF CELLULOSE IN SECONDARY WALLS OF NORMAL CONIFEROUS TRACHEIDS

In the case of the normal 3-layered tracheids of conifers (Pl. 208, Fig. 9), it is possible, by varying the details of the technique, to induce crystals to form (1) within the central layer of the secondary wall, (2) throughout both the central layer and the outer layer, or (3) in the outer layer alone. We have not succeeded, as yet, in obtaining them within the tenuous inner layer of the secondary wall. This appears to be due, at least in part, to the fact that the iodine escapes, during the treatment, from the exposed surfaces of the wall.

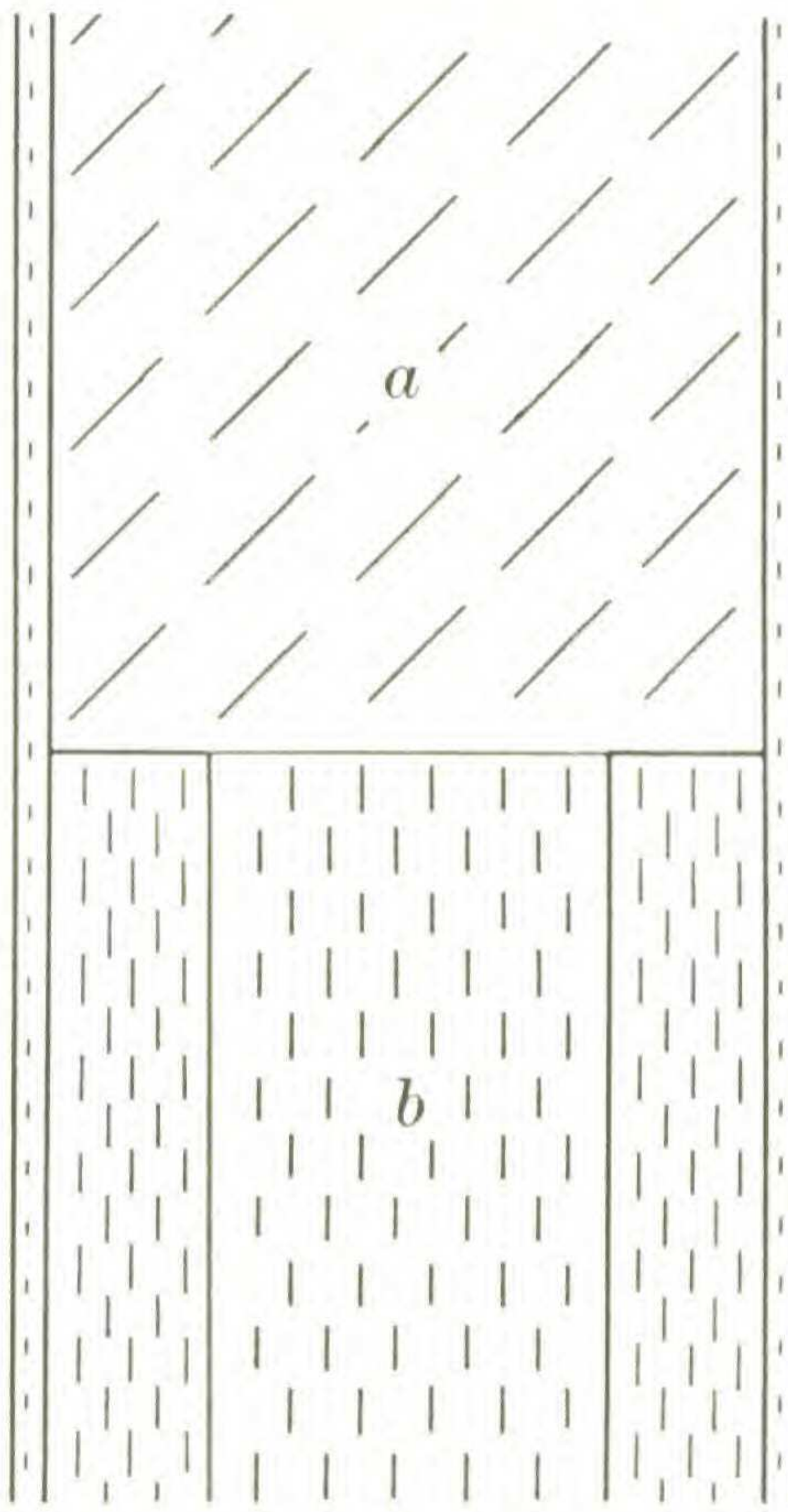
A. Orientations of the outer layer

The orientation of the cellulose in the outer layer of the secondary wall fluctuates more or less from specimen to specimen, from tracheid to tracheid of the same specimen, and in different parts of the same cell. In certain samples of wood that we have sectioned, the cellulose tends to be arranged at right angles to the long axis of the cell in the unpitted parts of the earlywood tracheids (Text fig. 1a; Pl. 206, Figs. 1 and 3), and to have a helical orientation in homologous parts of the latewood tracheids (Text fig. 2a; Pl. 206, Fig. 4, and Pl. 207, Figs. 5 and 6). In other samples of wood, the arrangement may be helical in both earlywood and latewood, or it may fluctuate from tracheid to tracheid throughout the annual ring. There is no evidence to indicate that specific orientations are characteristic of particular species. On the contrary, the available data suggest that the arrangement of the cellulose fluctuates considerably in different parts of the same tree.

Local deviations in the prevailing orientation of any specific tracheid are of common occurrence in pitted parts of the wall. Not only is there a circular arrangement of the cellulose in the embossed parts of the wall which form the borders of the pits (Text fig. 3), but there is a modified orientation in the adjacent parts of the wall as well. Such local deviations in orientation are more extensive and pronounced in large thin-walled tracheids than in small, thick-walled ones.



TEXT FIGURE 1



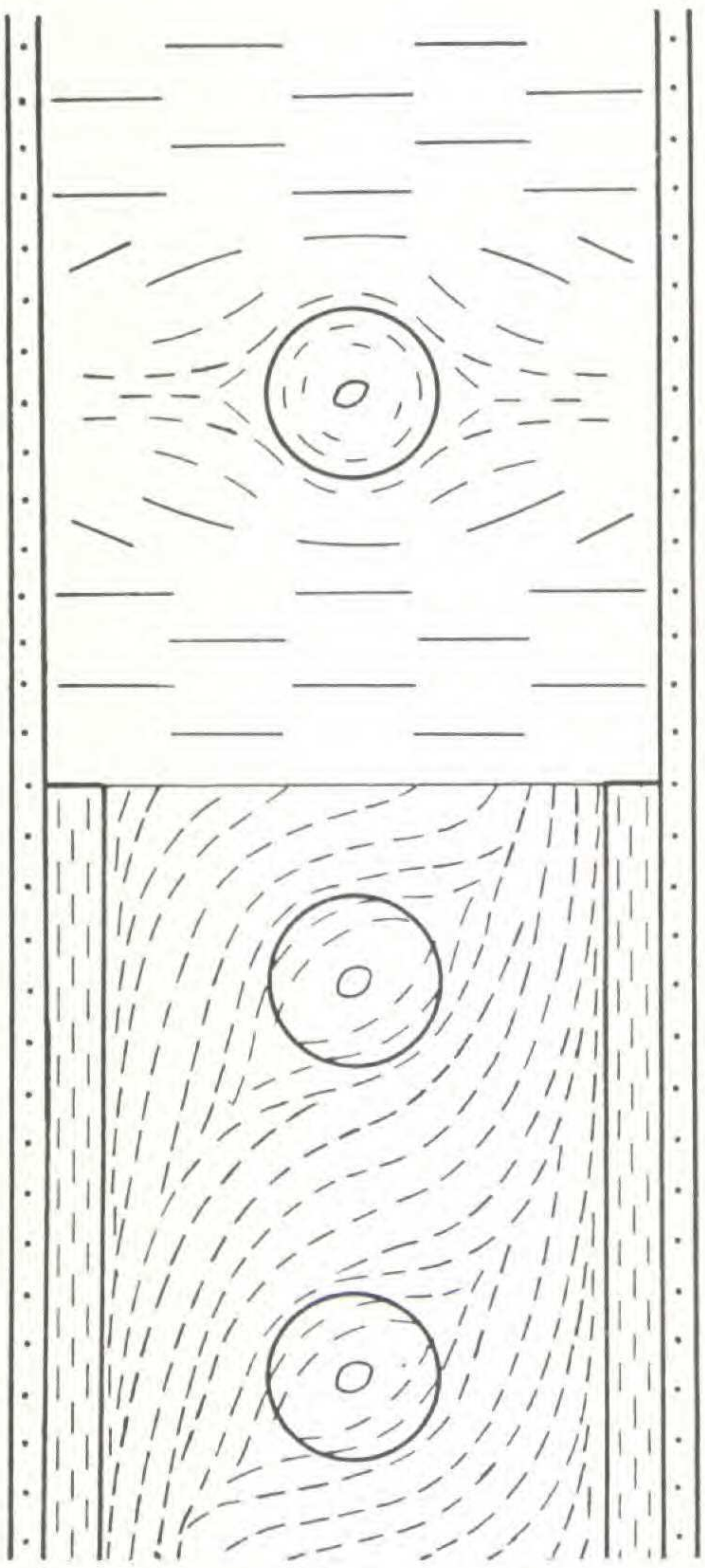
TEXT FIGURE 2

TEXT FIGURE 1. Diagrammatic illustration of the orientations of cellulose in the outer layer (a) and the central layer (b) of the unpitted tangential wall of a normal earlywood tracheid.

TEXT FIGURE 2. Diagrammatic illustration of the orientations of cellulose in the outer layer (a) and the central layer (b) of the unpitted tangential wall of a normal latewood tracheid.

B. ORIENTATIONS OF THE CENTRAL LAYER

The arrangement of the cellulose in the central layer of the secondary wall also varies more or less from specimen to specimen, from tracheid to tracheid, and in different parts of the same cell. Not infrequently the fibrils of cellulose tend to be oriented more nearly parallel to the long axis of the cell in the latewood (Text fig. 2b; Pl. 207, Fig. 5), than in the earlywood (Text fig. 1b; Pl. 206, Fig. 3), but helical arrangements are of not uncommon occurrence in latewood, and the cellulose may, at times, be oriented parallel to the long axis of the cell in earlywood. The most striking deviations in orientation occur in pitted parts of the wall, particularly in the radial walls of the earlywood tracheids (Text fig. 3; Pl. 206, Fig. 4). The fibrils do not have a



TEXT FIGURE 3. Diagrammatic illustration of the orientations of cellulose in the outer layer and the central layer of the pitted radial wall of a normal earlywood tracheid.

circular or concentric arrangement in the embossed parts of the wall — as is the case in the outer layer of the secondary wall — but curve about and through the borders of the pits as indicated in Text fig. 3. Thus, in the earlywood, the orientation of the cellulose commonly tends to deviate more from the vertical axis in the radial walls than in the unpitted or sparsely pitted tangential walls (Text figs. 1–3).

The orientation of the fibrils may fluctuate, at times, in the successively formed growth rings or lamellae of the central layer, but pronounced shifts in orientation are of relatively infrequent occurrence in the tracheids of conifers. Regularly recurring changes from right-handed to left-handed helixes or *vice versa*, such as are hypothesized by various investigators, are rarely, if ever, encountered in the central layer of coniferous tracheids.

C. Correlations between the outer layer and the central layer

There are four combinations of orientations that are of common occurrence in the secondary walls of normal coniferous tracheids:

(1) The cellulose of the outer layer may be oriented at right angles to the longitudinal axis of the cell, and the cellulose of the central layer may be arranged parallel to this axis.

(2) The cellulose of the outer layer may be oriented at right angles to the long axis of the cell, whereas that of the central layer has a helical arrangement.

(3) The cellulose of the outer layer may have a helical orientation, whereas that of the central layer is arranged parallel to the long axis of the cell.

(4) The cellulose of both the outer layer and the central layer may be helically oriented.

It is significant in this connection, however, that the helixes of the central layer have relatively steep slopes and rarely deviate as much as 45 degrees from the longitudinal axis of the cell, whereas those of the outer layer usually have comparatively low slopes. Thus, even when both layers have helical arrangements, the orientations rarely, if ever, are parallel. In all the material of normal coniferous tracheids that we have examined, the differences in orientation in unpitted parts of the secondary wall are of such magnitude that they may be detected when very thin (5 μ) transverse sections of the cells are examined in polarized light between crossed nicols. In other words, the central layer in such sections is either isotropic (Pl. 208, Fig. 9), or detectably less birefringent than the outer layer (Pl. 208, Fig. 13). This is due, of course, to the fact that a layer is dark in transverse sections when its cellulose is oriented parallel to the long axis of the cell but attains its maximum birefringence when the cellulose is oriented at right angles to this axis.

It should be noted, in passing, that according to Frey,[1] the micelles

[1]Frey, A., Die submikroskopische Struktur der Zellmembranen (Jahrb. Wiss. Bot. **65**: 195-223. 1926).

of cellulose have a circular orientation in the borders of the pits, whereas Scarth and his co-workers[1] consider that the fibrils merely curve about the bordered pits, "instead of regularly circling round them." Both investigators are right or wrong, depending upon the part of the wall which is selected for observation. As we have shown, the orientation of the outer layer is circular and thus in agreement with Frey's contention, whereas that of the central layer is entirely in accord with Scarth's view.

ORIENTATION OF CELLULOSE IN THE SECONDARY WALLS OF THE TRACHEARY CELLS OF DICOTYLEDONS

A. Normal 3-layered tracheids, fiber-tracheids, and libriform fibers

The arrangement of the cellulose in secondary walls of normal 3-layered tracheids, fiber-tracheids, and libriform fibers of dicotyledons resembles that which occurs in the tracheids of conifers. Thus, the orientation of the outer layer of the secondary wall fluctuates from positions at right angles to the longitudinal axis of the cell (Pl. 207, Fig. 6), to various helical arrangements, whereas that of the central layer varies from helical to longitudinal. It is significant, however, that with the progressive reduction of bordered pits in fiber-tracheids and libriform fibers, the orientation — particularly of the central layer — tends to show less extensive local deviations, and the arrangements of the cellulose in the radial and tangential walls are more nearly uniform and comparable.

B. Aberrant types of fiber-tracheids and libriform fibers

Deviations in the orientation of cellulose in the successively formed parts of the central layer appear to be of more frequent occurrence in thick-walled fiber-tracheids and libriform fibers of dicotyledons than in the tracheids of the latewood of conifers. Not infrequently the deviations are of such magnitude that they may be detected when thin transverse sections of the cells are viewed in polarized light between crossed nicols (Pl. 208, Fig. 10). In the so-called gelatinous fibers of dicotyledons, there are abrupt transitions from concentric to radial or radio-concentric structural patterns and *vice versa*. These abrupt changes in the structural pattern of the cellulosic matrix may or may not involve concomitant modifications in the orientation of the cellulose in relation to the longitudinal axis of the cell.

[1]Scarth, G. W., R. D. Gibbs, & J. D. Spier. The structure of the cell wall and the local distribution of the chemical constituents (Trans. Roy. Soc. Canada **5**: 269-279. 1929).

c. Vessels

In the less specialized types of dicotyledonous woods, the vessel members resemble tracheids in size, form, and structure. They tend to be comparatively thin-walled, and to have secondary walls which are conspicuously 3-layered, except in heavily pitted parts where the vessel members of the same or of different vessels are in contact. The arrangement of the cellulose in such 3-layered secondary walls of vessels (Pl. 208, Fig. 11), fluctuates much as it does in normal tracheids. Thus, the outer layer may have an orientation at right angles to the long axis of the cell, or it may have a helical arrangement of comparatively low slope. As in the case of normal tracheids, the central layer has an orientation which is either parallel to the long axis of the cell or steeply helical.

As the vessels of dicotyledons become more and more highly specialized, they tend to form secondary walls of a wider range of structural complexity and diversity. Not infrequently, they tend to lose their typical 3-layered structure and to form multi-layered walls or thick walls which are more or less birefringent throughout (Pl. 208, Fig. 14), when transverse sections are viewed in polarized light between crossed nicols. In other words, the orientation of the cellulose in the more highly specialized types of dicotyledonous vessels frequently deviates markedly from that which occurs in normal tracheids.

It should be emphasized, in this connection, that variations in the thickness of the secondary walls of normal 3-layered tracheids are due primarily to variations in the width of the central layer (Pl. 208, Fig. 9), — i. e., of a layer which is dark or feebly birefringent in transverse sections. On the contrary, fluctuations in thickness of the secondary wall of vessels commonly are due to variations in the width of layers which are intensely birefringent (Pl. 208, Figs. 12 and 14), when transverse sections are examined in polarized light between the crossed nicols. Thus, the more conspicuous differences between the secondary walls of tracheids and of specialized types of vessel members are due primarily to different orientations of cellulose in the successively formed parts of the secondary wall.

DISCUSSION

The secondary wall of tracheary cells and fibers is composed of a continuous and firmly coherent matrix of anastomosing fibrils of cellulose. Lignin and other non-cellulosic substances may be deposited in the elongated, intercommunicating interstices of this matrix, thus resulting in two continuous interpenetrating systems of different chemical composition. The threadlike parts of the two interpenetrating systems have parallel orientations. Therefore, the crystals of iodine which form within the elongated interstices of the cellulosic matrix after chlorination are oriented parallel to the long axis of the fibrils of cellulose. That such is indeed the case may be demonstrated by various corroborative lines of evidence.

(1) Where aggregations of fibrils or so-called striations are clearly visible in surface views of unswollen secondary walls, the crystalline aggregates of iodine are oriented parallel to the long axes of these structures.

(2) In the case of thick secondary walls of the 3-layered type — where the slitlike pit orifices are oriented parallel to the fibrillar axis of the central layer — the crystals of the central layer are arranged parallel to the slitlike orifices of the pits.

(3) In secondary walls having a pronounced radio-longitudinal or radio-helical structural pattern — where mechanical cleavages and seasoning cracks are oriented longitudinally or radio-helically — the crystals of iodine are arranged parallel to the cleavage planes and seasoning cracks.

(4) In favorable material — where the angles of extinction and dichroic phenomena in polarized light are clearly visible and can be accurately measured — the orientation of the cellulose as determined by the crystal method is in close agreement with the data obtained by polarization techniques. In fact, the evidence secured by the crystal and polarization techniques is so strikingly complementary that our photographs of crystal orientations might be substituted for Frey's diagrams of micellar arrangements in homologous layers.

The crystal method is so significant in studying the details of fibrillar orientation in different parts of a single wall or layer that it is of interest to determine whether the technique may be modified for use in the study of unlignified secondary walls. This has been attempted by Doctor Thomas Kerr, who has succeeded in inducing crystals of iodine to form within the wall of the cotton hair. His modification of our technique consists in staining the secondary wall of the cotton hair with iodine-potassium iodide or with chloroiodide of zinc, and subsequently treating the stained preparation with a syrupy, "supersaturated" solution of zinc chloride. Thus, it is evident that the crystal method may be modified for the study of both lignified and unlignified secondary walls.

CONCLUSIONS

1. Crystalline aggregates of iodine may be induced to form within the elongated interstices of the cellulosic matrix of the secondary wall. These elongated crystals are oriented parallel to the long axis of the fibrils of cellulose and therefore of the micelles and chain molecules.

2. The crystalline aggregates are so conspicuous and so clearly visible, microscopically, that it is possible not only to detect such major variations in orientation of the cellulose as occur in passing from layer to layer of the secondary wall, but also to observe such fluctuations in orientation as occur within the limits of a single layer.

3. In the case of normal, 3-layered tracheids, fiber-tracheids, and libriform fibers, the orientation of the cellulose in the outer layer and in the central layer of the secondary wall fluctuates more or less from specimen to specimen, from cell to cell, and in different parts of the same cell.

4. Although the orientation of the cellulose may deviate, at times, in the successively formed growth rings or lamellae of the central layer, there is no regular alternation of right-handed and left-handed helixes as hypothesized by various investigators.

5. In the case of the large bordered pits of the earlywood of conifers, the cellulose has a circular orientation in the outer layer, but merely curves about the pits in the central layer.

6. The less specialized types of dicotyledonous vessels resemble normal tracheids in having a 3-layered secondary wall, whereas the more highly specialized types have walls of a much wider range of complexity and structural variability, owing to fluctuations in the orientation of the cellulose.

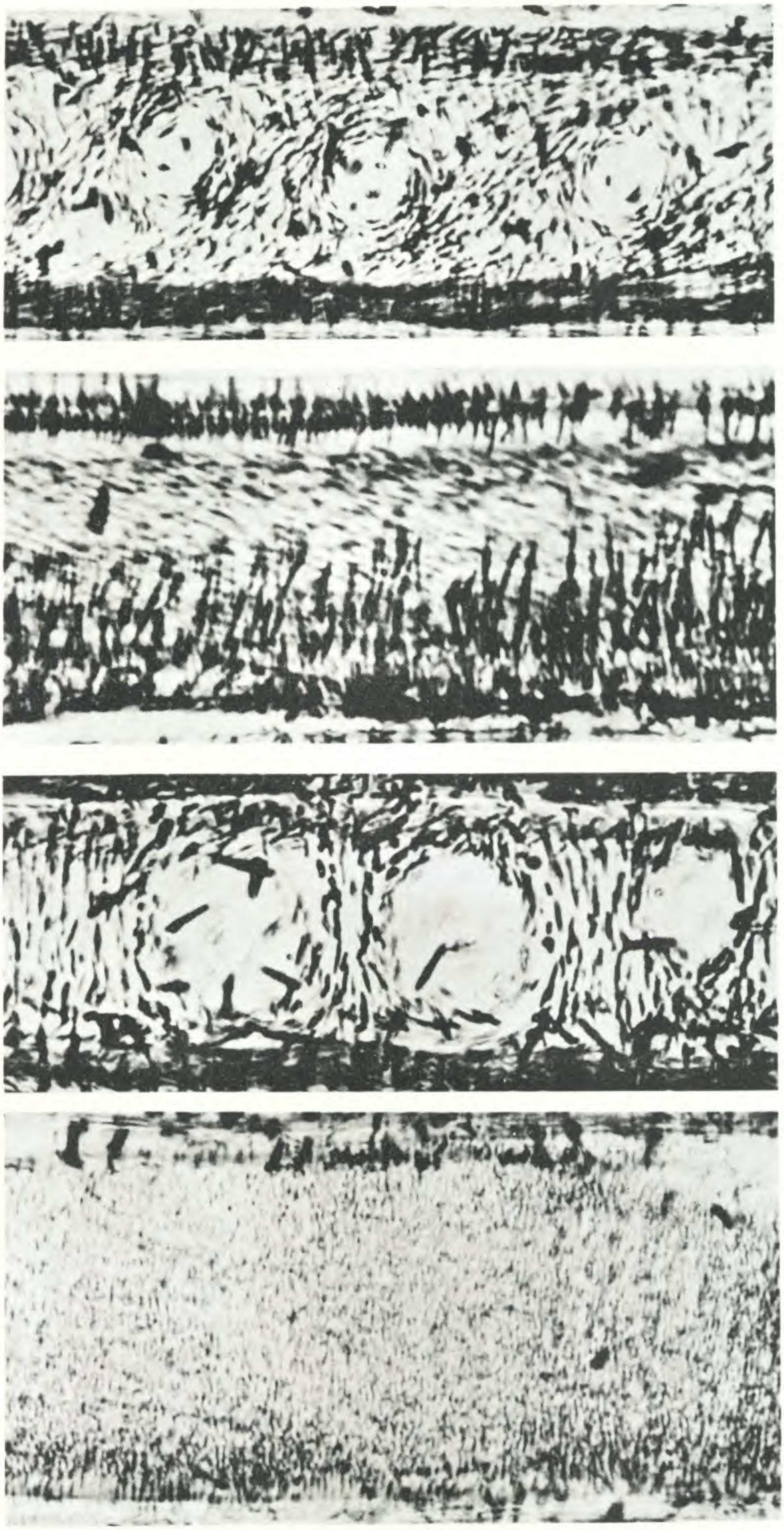
DESCRIPTION OF PLATES

PLATE 206

Fig. 1. *Sequoia sempervirens* Endl. Unpitted radial wall of earlywood tracheid, showing, in the outer layer, numerous minute crystals oriented at right angles to the long axis of the cell. × 750.

Fig. 2. *Sequoia sempervirens*. Pitted radial wall of earlywood tracheid, showing, in the outer layer, coarser crystalline aggregates oriented at right angles to the long axis of the cell except in proximity to the bordered pits. The larger irregularly oriented crystals are lying upon the exposed inner surface of the wall. × 750.

Fig. 3. *Sequoia sempervirens*. Earlywood tracheid from which part of the outer layer has been cut away (right), showing transverse orientation of crystals in the outer layer of the secondary wall (left) and steeply helical arrangement in the central layer (right). × 1000.



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2

1

Orientation of Cellulose

FULL-TONE—MERIDEN